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Introduction

The Workshop on Lyme Borreliosis was held on 18 June 1990 in Stockholm during the Fourth International Conference on Lyme Borreliosis.

The aim was to follow up two previous meetings (Baden, Austria, 1987 and Prague, 1989) and to continue discussing standardized Lyme borreliosis serology.

Discussion

The principal investigator, Dr Jan Jirous, presented data from the European interlaboratory comparative study, which began after the 1989 meeting in Prague.

Twenty-six laboratories in 14 countries performed a blind analysis for anti-Borrelia burgdorferi IgG on five well characterized serum samples. A high level of agreement was achieved; only the results of one serum sample disagreed significantly. The sample in question was a low positive, from a patient with chronic neuroborreliosis. Seven of the 26 laboratories rated it negative, 6 called it borderline and 13 found it positive. Five laboratories also analysed 96 randomly selected blood-donor sera from Prague for anti-Borrelia IgG antibodies. This was to assess whether the cut-off level used at these laboratories was comparable and whether antibody prevalence in a population depends on the area of origin. All five laboratories said they had used a test-specificity of 98% (which means an expected sero-positive frequency among healthy controls of 2%). The study revealed significant differences: three laboratories found 4 positives out of 96, and the other two found 11 and 8 respectively.

The workshop participants agreed that the interlaboratory variations were due mainly to minor differences in the assay cut-off levels used. The fact that the laboratories disagreed on only one low-positive sample was a good sign; nevertheless it creates a significant problem, since in routine tests many positive samples have antibody levels in this critical area. The quite different numbers of seropositives found by the various laboratories among the Prague blood-donor samples indicating that they used different assay cut-off values is also important, because this value will significantly influence the number of possible "false-positive" test results. However, the study shows that interlaboratory agreement has improved significantly since the first workshop in Baden, Austria, in 1987 (see report EUR/ICP/CDS 011, Table 1).^a

As to the data on the Prague blood-donor sera, the prevalence of anti-B. burgdorferi antibodies proved to be similar in randomly selected healthy controls from different geographical areas. Certain subpopulations known to have a high exposure to ticks (such as forestry workers) may show a higher antibody prevalence to B. burgdorferi. This means that it should be possible to use the same diagnostic cut-off for serological assays done in different European countries. Large numbers (200-500) of blood donors (representing healthy controls) need to be examined to assess the general antibody prevalence, which must be used to define a diagnostic cut-off.

The participants discussed the possible significance of antigenic differences in B. burgdorferi strains used as serological test antigens. The general opinion was that in the case of individual serum samples, especially those with borderline and low antibody levels, different results might be obtained with different strains. But when many samples are being investigated (as is the case in routine laboratories), the choice of strain for

^a Workshop on Lyme borreliosis: report on an International Meeting, Copenhagen, WHO Regional Office for Europe, 1987 (unpublished document EUR/ICP/CDS 011).

test-antigen preparation is not important. It can be difficult to reproduce the quality of a test antigen from a single strain due to variable culture conditions, sonification efficiency and partial extraction of the crude spirochetal sonicate, so the choice of strain probably plays a minor role.

The need to use regional collected strains as test antigen was discussed. According to the above-mentioned arguments for antigen preparations and the fact that a diversity of different strains may be found within the same region, the general opinion was that this is not necessary.

Participants discussed using purified spirochetal antigens or protein fractions as test antigens. As yet the only candidates are the p41 flagellar protein, the pC protein of 23 kD and the so-called fraction B (a spirochetal cell extract depleted of p41 and enriched for the osp A-B proteins). Experience is limited to the p41 flagellar assay, which compares favourably with conventional whole-cell extracts. Opinions on p41 differ and not everyone yet has the faith to rely on a single protein as a test antigen. However, one participant (Dr Bettina Wilske, Munich) did add weight to the theory that use of a single antigen may improve Lyme serology. She briefly presented very recent data, comparing samples from 75 patients with erythema migrans. The samples were subjected to different ELISAs, using either/or a traditional sonic extract, a commercial kit using purified native p41 flagellar protein, recombinant osp A protein, a recombinant pC protein, a recombinant p41 protein and a combination of recombinant pC and recombinant p41 protein. The results were as follows (seropositive=diagnostic sensitivity):

Sonic extract	25.5%
Purified native p41	38.7%
Recombinant osp A	9.3%
Recombinant pC	24.7%
Recombinant p41	29.3%
Recombinant pC and p41	45.3%

For lack of time, the remaining agenda items were not discussed.

Conclusions

1. A European interlaboratory study showed minor but important differences, due mainly to different assay cut-offs.
2. The prevalence of anti-B. burgdorferi antibodies in the unselected general population seems similar in different European countries. This should allow investigators to use the same, comparable cut-off level.
3. In terms of protein profile or area of origin, the choice of B. burgdorferi strain for test-antigen preparation is of minor importance for large-scale routine serology.
4. In future, Lyme serology may be improved by using purified native or recombinant Borrelia antigens, either singly or in combination.

Annex I

THE EUROPEAN INTERLABORATORY STUDY

by

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(principal investigator)

The aim of this study was to compare serological results obtained by various European laboratories investigating standard sera. The study was divided into two parts.

- In the first part, three sera were collected from Lyme Borreliosis patients and two from controls (Table 1). The serum samples were lyophilized per 0.5 ml and distributed at the beginning of 1990 to 30 European laboratories in 14 countries to test for the presence of specific IgG Borrelia antibodies.

Table 1. Interlaboratory study: IgG antibodies
(five standard sera)

1. Neuroborreliosis (cranial neuritis), during therapy
2. Neuroborreliosis (MS-like demyelinated disease), after treatment
3. Control
4. Acrodermatitis chronica atrophicans, after treatment
5. Control

- In the second part, five laboratories performed a serological investigation of 96 sera from Prague blood donors. The laboratories of Dr Wilske (Munich), Dr Wright (London), Dr Hansen (Copenhagen), Dr Stiernstedt (Stockholm) and Dr Jirous (Prague) took part. A second purpose was to compare the occurrence of positive serological findings in blood donors' sera from Czechoslovakia and from other countries.

Twenty-six laboratories in 14 European countries investigated the five standard sera. Most work with ELISA and IFA, others with an IHA test. The results (shown in Table 2) came from original procedures of the participating laboratories using various strains of B. burgdorferi to prepare antigens: some of the laboratories used commercial tests. An agreement of 100% was achieved in the results for sera nos. 1, 3, 4 and 5. A disagreement in serological results occurred only in one patient (No. 2) who had chronic neuroborreliosis. In 13 laboratories, the result for this patient was positive, in 6 it was a weak positive (borderline titre), and in 6 the value found was under positivity level (cut-off).

The results of the second part of the study - investigation of the Czechoslovak blood donors' sera - are shown in Table 3. Of 96 sera, 17 were found IgG-positive by one laboratory at least. The greatest number of positives (17) was found in the Czechoslovakian laboratory. In 14 of these positive sera, the specific IgG antibodies were also detected by one of the other laboratories. Complete (100%) agreement by all five laboratories on IgG positivity was seen for only three sera (Nos. 4, 60 and 94). Comparison of the presence of IgG antibodies in Czechoslovak blood-donors' sera and those of Danish and German blood donors suggests a similar incidence of antiborrelial IgG antibodies in a healthy population in Czechoslovakia and Germany. In Denmark the incidence seems to be slightly lower.

Table 2. Interlaboratory study:
IgG antibodies (five standard sera)

Laboratory	Sera					Method
	1	2	3	4	5	
Belgium (Dr Bigaignon)	+	-	-	+	-	ELISA, IFA
Belgium (Dr Goubau)	+	-	-	+	-	ELISA, IFA(Ig conjugate)
Czechoslovakia (Dr Jirous)	+	+	-	+	-	ELISA (<u>B. recurrentis</u>) ELISA (<u>B. burgdorferi</u>)
Denmark (Dr Hansen)	+	+	-	+	-	ELISA (Flagellum)
France (Dr Postic)	+	-	-	+	-	ELISA, IFA
Germany (Dr Ackermann)	+	+	-	+	-	ELISA, IFA
Germany (Dr Peters)	+	+	-	+	-	ELISA
Germany (Dr Wilske)	+	(+)	-	+	-	ELISA, IFA
Hungary (Dr Bozsik)	+	(+)	-	+	-	ELISA (Detecta-Dot), IHA (Diagast)
Hungary (Dr Lakos)	+	(+)	-	+	-	IFA
Italy (Dr Cinco)	+	+	-	+	-	ELISA
Netherlands (Dr Nohlmans)	+	+	-	+	-	ELISA, IFA
Netherlands (Dr Rijpkema)	+	-	-	+	-	ELISA
Netherlands (Dr Spanjard)	+	-	-	+	-	IFA (Ig conjugate)

Table 2 (contd)

Laboratory	Sera					Method
	1	2	3	4	5	
Norway (Dr Jenum)	+	+	-	+	-	ELISA
Norway (Dr Melby)	+	+	-	+	-	IHA (Diagast)
Sweden (Dr Granstrom)	+	0	-	+	-	ELISA
Sweden (Dr Hederstedt)	+	+	-	+	-	ELISA (Whole-cell) ELISA (Flagellum)
Sweden (Dr Kaijser)	0	+	-	+	0	IFA
Sweden (Dr Stedingk)	+	+	-	+	-	ELISA
Sweden (Dr Stiernstedt)	+	(+)	-	+	-	ELISA
Switzerland (Dr Gern)	+	+	-	+	-	ELISA, IFA
USSR (Dr Korenberg)	+	(+)	-	+	-	IFA
United Kingdom (Dr Cutler)	+	(+)	-	+	-	ELISA (Ig conjugate)
United Kingdom (Dr Zochovski)	+	+	-	+	-	ELISA (Dakopatts, flagellum), IFA
Yugoslavia (Dr Lako)	(+)	-	-	(+)	-	IFA (Virion)

Table 3. Interlaboratory study: IgG antibodies, 96 blood donors' sera (Prague)

Positive sera	ELISA- with test antigen as indicated				
	Czechoslovakia (Dr Jirous)	United Kingdom (Dr Wright)	Germany (Dr Wilske)	Denmark (Dr Hansen)	Sweden (Dr Stierstedt)
(number)	<u>B. burgdorferi</u> sonicate	<u>B. burgdorferi</u> sonicate	<u>B. burgdorferi</u> sonicate	<u>B. burgdorferi</u> purified flagellum	<u>B. burgdorferi</u> sonicate
2	- ^a	-	-	-	-
4	+ ^c	+	(+)	+	+
45	(+)	+	-	-	-
50	-	-	-	-	(+)
55	-	-	-	-	(+)
56	-	(+)	-	+	-
59	(+)	+	+	+	-
60	+	+	+	+	+
62	-	+	-	-	-
83	-	+	(+)	-	+
85	+	(+)	+	+	+
94	+	+	+	+	+
98	-	(+)	-	-	-
112	(+)	(+)	-	-	(+)
114	-	(+)	-	-	-
153	-	+	(+)	+	-
154	-	+	+	+	-
155	-	+	+	+	-
156	-	(+)	-	-	-
	3(3)	11(6)	8(4)	4(6)	4(3)

^a Negative.

^b Sorderrine, judged as positive, using the 95% percentile as cut-off.

^c Positive, using the 98% percentile as cut-off.

I should like to add some personal remarks to the results of the study. A very good agreement was achieved in the results on the five sera. The disagreement on serum No. 2 was caused mainly by different cut-off levels. Similar results were presented at our previous meeting in Prague on Interlaboratory Study I. There was total agreement on IgG antibodies in 8 out of 10 sera from Lyme borreliosis patients investigated by five laboratories.

The greater differences in the blood-donors' sera results might also have been caused by the use of various antigens in the serological methods. Similar results from an investigation by four United States' laboratories of 132 serum samples from outdoor workers were published by Schwartz et al. in 1989*.

The Interlaboratory Study was organized in 1989, has been performed in 1990 and is now being evaluated. But one big question remains: can some data help us in our attempts at standardization? I believe so. Preparation of the antigen from a standard B. burgdorferi strain and an agreed cut-off level are necessary conditions for starting effective international cooperation between laboratories.

* Schwartz, B.S. et al. Antibody testing in Lyme disease. A comparison of results in four laboratories. Journal of the American Medical Association, 262: 3431-3434 (1989).

Annex 2

LIST OF PARTICIPANTS

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